3 ug/ml) against oxidized low-density lipoprotein (ox-LDL)-induced Human umbilical vein endothelial cells (HUVECs) dysfunction in vitro.

METHODS In vivo, obesity-related hypertensive rat models were induced by high-fat diet for 25 weeks. The aqueous extract of TT and telmisartan was intragastrically administrated for 8 weeks. Body weight, blood pressure and heart rate were measured weekly to observe the slimming benefits and anti-hypertensive effects of the drugs. The endothelial morphology of the thoracic aorta was observed by HE staining and scanning electron microscope. The level of serum lipid was measured by biochemical methods, and serum leptin, AngII, ET-1, NO, NPY and Hcy was determined by ELISA. In vitro, HUVECs were pre-incubated for 60 min with TT (30 ug/ml and 3 ug/ml separately) or 10-5 mol/l telmisartan and then the injured endothelium model was established by applying 100 μg/ml ox-LDL for 24 h. Cell viability of HUVECs was observed by real-time cell electronic sensing assay and apoptosis rate by Annexin V/PI staining. The cell migration assay was performed with a Transwell insert system. Cytoskeleton remodeling was observed by immunofluorescence assay. The content of eNOS was measured by ELISA. Intracellular reactive oxygen species (ROS) generation was assessed by immunofluorescence and flow cytometer. Key genes associated with the metabolism of ox-LDL were chosen for quantitative real-time PCR to explore the possible mechanism of TT against oxidized LDL-induced endothelial dysfunction.

RESULTS TT decreased systolic pressure, diastolic pressure, mean arterial pressure and heart rate, and showed against weight gain effect. TT improved endothelial integrity of thoracic aorta, decreased leptin, AngII, ET-1, NPY and Hcy, while increased NO.

In vitro, TT suppressed ox-LDL-induced HUVEC proliferation and apoptosis rates significantly and TT prolonged the HUVEC survival time and postponed the cell's decaying stage. TT improved the endothelial cytoskeletal network and increased cell migration. Additionally, TT regulated of the synthesis of endothelial nitric oxide synthase and generation of intracellular reactive oxygen species. TT significantly decreased mRNA expression of PI3Kα and Socs3. It also increased mRNA expression of Akt1, AMPKα1, JAK2, LepR and STAT3 induced by ox-LDL. The results suggested that the JAK2/STAT3 and/or PI3K/AKT pathway may be a very important pathway of the pharmacological mechanism of TT against endothelium injury.

CONCLUSIONS TT demonstrated excellent slimming benefits, anti-hypertension and endothelial protective effects. It also suggested that the JAK2/STAT3 and/or PI3K/AKT pathway might be a very important pathway which was involved in the pharmacological mechanism of TT as the vascular protective agent.

GW26-e3603

Cardiac Fibroblast Contributes to Myocardial Fibrosis in Mice With Diabetes Mellitus-Role of Cardiomyocyte-Fibroblast Interaction

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OBJECTIVES The major cardiac cell type expressing HMGB1 is the cardiac myocyte (CM) while the primary IL-33 expressing cell is the cardiac fibroblast (CF). Here we delineated the extracellular communication pathway between cardiomyocyte and fibroblast that contributes to murine DiCM.

METHODS DM was induced in 6-week-old, male C57BL/6 mice by intraperitoneal (i.p.) injection of streptozotocin (STZ). The sex matched littermates were injected with an equal volume of citrate buffer as controls (pH=4.5). CMs or CFs cultured individually or in co-culture were challenged with 30 mM glucose in M199 to simulate the hyperglycemia of DM. As an osmotic control, the cells were incubated with 30 mM mannitol in M199. Immunoflourescence staining and Western blot were used for assessment of protein expression of HMGB1, IL-33 and collagen I and III. A mouse pressure-volume loop analysis system was used for assessment of myocardial function.

RESULTS 1) myocardial expression of HMGB1 and IL-33 were detected by immunoflourescence staining and Western blot in the murine STZ model of DM. Myocardial expression of HMGB1 was increased, while that of IL-33 was decreased at 2 and 4 weeks after achieving a hyperglycemic state. HMGB1 was primarily localized to the myocytes, while IL-33 was localized to the interstitial fibroblasts.

2) Mice developed cardiomyopathy six weeks after the induction of DM as indicated by increased myocardial fibrosis and dysfunction. The myocardial collagen deposition and improvement of myocardial function were substantially attenuated by inhibition of HMGB1or exogenous IL-33.

3) Although challenge of isolated CFs with HG increased HMGB1 production, the effects were minimal compared to those noted in CM. In vitro HG model, cardiac myocytes can potentiate the down-regulation of IL-33 in CFs; an effect mediated by myocyte-derived HMGB1.

4) HG challenge of CFs alone slightly increased collagen I expression. The effect was significantly enhanced when CFs were co-cultured with CMs; the potentiating effect was abrogated by the HMGB1 inhibitor, A-box. Further, increase in collagen I expression by isolated CFs in response to HG, was potentiated by exogenous administration of HMGB1.

5) When $TRL4^{-/-}$ CFs were co-cultured with wild type CMs, the CM-induced potentiating effect on down-regulation of CF IL-33 and increase in CF collagen production was negated. Two weeks after the induction of the STZ model, the expected decrease in IL-33 was noted in WT mice, but ,it was not evident in $TLR4^{-/-}$ mice. Further , in $TLR4^{-/-}$ mice, the STZ-induced myocardial fibrosis and dysfunction were blunted.

CONCLUSIONS Our data support that cardiac myocyte-fibroblast interaction plays a key role in diabetic myocardial fibrosis. Specifically, our study indicates myocyte HMGB1-fibroblast TLR4/IL-33 axis contributes to the development of myocardial fibrosis and dysfunction in mice with diabetes.

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GW26-e3847

Postinfarction Gene Therapy With Hepatocyte Growth Factor Mitigates Cardiac Remodeling and Dysfunction

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OBJECTIVES To investigated beneficial effects and its mechanisms of naked plasmid expressing recombinant human hepatocyte growth factor on left ventricular remodeling and dysfunction.

METHODS Acute myocardial infarction was induced male SD rats by ligating anterior descending of left coronary artery. These rats were randomly assigned to HGF group (n=8); a single myocardial injection of naked plasmid expressing HGF (250 ug/injection) immediately after left coronary artery ligation. Control group (n=8);myocardial injection of same dose naked plasmid without HGF, normal group (n=10); the suture was passed but not tied treated. After four or eight weeks. cardiac function was evaluated by echocardiography respectively, the cardiacs specimens at eight-week time point were subjected to Masson staining and immunohistochemical analysis.

RESULTS Four weeks later, left ventricular remodeling and dysfunction were apparent,and LV anterior wall thickness (LVAWT) were significantly reduced ($P<0.001$) in the control group. However, left ventricular remodeling and dysfunction were significantly relieved ($p<0.05$) and LVAWT were thicker ($p=0.378$) in the HGF group. Eight weeks later, HGF-treated rats showed that left ventricular remodeling and dysfunction were still significantly improved ($p<0.05$), furthermore ,significant mitigation of LVAWT was seen in HGF-treated rats ($P<0.05$). Eight weeks later, the infarct size significantly reduced and the infarct wall was thicker in the HGF-treated rates ($P<0.05$). Myocardial fibrosis was significantly reduced and the density of blood capillary was significantly increased in the myocardial infarcted area in HGF group ($P<0.001$)

CONCLUSIONS Recombinant human hepatocyte growth factor improves postinfarction cardiac remodeling and dysfunction by reducing infarct size and myocardial fibrosis and increasing by density of blood capillary.

GW26-e3875

Diabetes Blunt the Compensatory Enhancement of SUMOylation Intensity of Sarcoplasmic Reticulum Calcium-transporting ATPase After Myocardial Infarction

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OBJECTIVES Diabetes is an independent risk factor of heart failure and mortality after myocardial infarction(MI). The activity and

expression of Sarcoplasmic Reticulum Calcium-transporting ATPase (SERCA2a) decrease in diabetes, leading to diastolic and systolic dysfunction of myocardium. It was recently reported that SUMOylation could elevate the activity and stability of SERCA2a. We assume that diabetes might affect the intensity of SUMOylation of SERCA2a after MI.

METHODS Diet-induced type 2 diabetic rats and controls were divided into suture ligation induced MI groups or sham groups. Primary cardiomyocytes were cultured in different concentrations of glucose and insulin, and underwent oxygen deprivation(OD) for 6 or 12 hours. Echocardiograph and left ventricular pressure were measured to determine cardiac function. The intensity of SUMOylation of SERCA2a, expressions of SERCA2a, SUMO1 and enzymes of SUMOylation were evaluated.

RESULTS Diabetes exacerbated diastolic and systolic dysfunction of myocardium after infarction. SUMOylation intensity of SERCA2a was enhanced in 1-week-post-MI non-diabetic rats and 6-hour-OD cardiomyocytes but not in 4-week-post-MI rats and 12-hour-OD cardiomyocytes. The expression of enzyme 2 of SUMOylation, namely Ubc9, was in accordance with the SUMOylation intensity, while SUMO1 and enzyme 1 were not changed. This compensatory enhancement was almost completely blunted in 1 or 4 weeks post-MI diabetic rats. Interestingly, glucose alone increased Ubc9 expression and the SUMOylation intensity of SERCA2a of cardiomyocytes in vitro in a concentration-dependent manner; however, with addition of insulin, glucose decreased Ubc9 and SUMOylation intensity in a concentration-dependent manner on the contrary, while SUMO1 and enzyme 1 were also not changed. Additionally, overexpression of Ubc9 with lentivirus neutralized the decreasing of SUMOylation intensity caused by glucose and insulin in vitro.

CONCLUSIONS SUMOylation intensity of SERCA2a was compensatory enhanced in post-MI non-diabetic rats, but not in diabetic rats. SUMOylation intensity of SERCA2a decreased in cardiomyocytes with addition of high glucose and insulin in vitro, which could be neutralized by overexpression of Ubc9. These observations provide evidence that Ubc9 and SUMOylation of SERCA2a is involved in diabetes-mediated exacerbation of left ventricular dysfunction after MI.

GW26-e3918

DCPIB Attenuates Myocardial Ischemia/Reperfusion Injury Through Inhibiting Autophagy in Rat Model

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OBJECTIVES Autophagy plays a contradictory role in myocardial ischemia/reperfusion (I/R) process, which acts as both beneficial cardioprotective during ischemia stress and myocardial injury in response to subsequent reperfusion. DCPIB (4-[(2-Butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-1-oxo-1H-inden-5-yl)oxy] butanoic acid), a selective inhibitor of volume-sensitive outwardly rectifying (VSOR) chlorine channel, has been determined to protect cardiomyocytes from reperfusion damage. However, the underlying mechanism remains unclear. The present study explored the possible mechanism of DCPIB in alleviating myocardial I/R injury.

METHODS Sprague-Dawley rats were randomly divided into sham operation group, I/R group, I/R+Rapamycin (RAPA) group, I/R+DCPIB group, I/R+3-methyladenine (3MA, a autophagic inhibitor) group and I/R+RAPA+DCPIB group, with 6 rats in each group. Rats were performed to ischemia 30 minutes and subsequent reperfusion 24 hours, DCPIB (10mg/kg), RAPA(4mg/kg) and 3MA (1mg/kg) were administered as intraperitoneal injection 10 min before the onset of reperfusion, respectively. Serum myocardial enzymes were measured and light microscopic study was performed, the myocardial LC3 was detected by immunohistochemistry, nuclear factor-κB (NF-κB) and tumor necrosis factor α (TNF-a) were detected by enzyme-linked immunosorbent assay (ELISA).

RESULTS The expressions of myocardial LC3, TNFa and NF-κB were significantly increased ($P<0.05$), and cardiac function was declined in I/R group compared with that in sham operation group ($P<0.05$), myocardial LC3 were furthermore increased in RAPA group, whereas these could be reversed through 3MA, a autophagic inhibitor. Of note, DCPIB administration caused significant reduction of myocardial LC3, TNF-a and NF-κB ($P<0.05$), and significantly improved the cardiac functional recovery and reduced myocardial enzymes activity compared with that in I/R group and I/R+RAPA+DCPIB group ($P<0.05$). Specific details for hemodynamics, compared to sham-operated group, left ventricular systolic pressure (LVSP), maximal rate of increase of ventricular pressure ($+dp/dt_{max}$) and maximal rate of decrease of ventricular pressure ($-dp/dt_{max}$) decreased while left ventricular end diastolic pressure (LVEDP) significantly increased markedly in I/R group and I/R+RAPA group ($P<0.05$), however, DCPIB indeed reversed the disorder.

CONCLUSIONS Our results demonstrated that DCPIB attenuated excessive autophagy to protect rat heart from I/R injury.

GW26-e3941

Urokinase Receptor Accelerates Ox-LDL Uptake and Foam Cell Formation by Upregulating CD36 Expression on Macrophages

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OBJECTIVES To investigate the role of uPAR in ox-LDL uptake and the interaction of uPAR and CD36 in ox-LDL internalization by macrophages.

METHODS THP-1 cells transfected with uPAR complementary DNA (uPAR overexpression THP-1, UOT) or with uPAR shRNA (uPAR low-expression THP-1, ULT) were established. Laser Scanning Confocal Microscope and dil-ox-LDL were used to evaluate ox-LDL uptake capacities. Oil red O staining was performed to measure lipid accumulation and foam cell formation. Cytokine expression levels of cytokines were quantitated by Real-Time-PCR.

RESULTS Ox-LDL-induced uPAR expression was independent of its baseline expression level in macrophages. UOT cells showed a strong correlation of uPAR expression with ox-LDL uptake. Much earlier dil-ox-LDL internalization and significantly more lipid droplets were found in UOT cells. Stable expression of a shRNA targeting uPAR gene strongly inhibits lipid uptake by macrophages. UOT cells exhibited significantly higher transcriptional levels of CD36 mRNA upon stimulation of ox-LDL.

CONCLUSIONS uPAR accelerates ox-LDL uptake and foam cell formation in macrophages, which may be mediated by CD36 up-regulation. The interaction between uPAR and CD36 may be a potential new therapeutic target.

GW26-e3962

Activation of D4 Dopamine Receptor Decreases AT1 Angiotensin II Receptor Expression in Rat Renal Proximal Tubule Cells

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OBJECTIVES The dopaminergic and renin angiotensin systems interact to regulate blood pressure. Disruption of the D_4 dopamine receptor gene in mice produces hypertension that is associated with increased renal AT_1 receptor expression. We hypothesize that D_4 receptor can inhibit AT_1 receptor expression and function in renal proximal tubules (RPTs) cells from Wistar-Kyoto (WKY) rats, the regulation of D_4 receptor on AT_1 receptor is aberrant in RPT cells from spontaneously hypertensive rats (SHRs).

METHODS The AT_1 receptor protein expression is detected by immunoblotting. Na^+-K^+-ATPase activity was determined as the rate of inorganic phosphate released in the presence or absence of ouabain.

RESULTS The D_4 receptor agonist, PD168077, ***decreased*** AT_1 receptor protein expression in a time (0-30hrs)- and concentration (10^{-9}-10^{-5}M)-dependent manner in WKY RPT cells. In contrast, in SHR cells, PD168077 (10^{-6}M) increased AT_1 receptor protein expression. The inhibitory effect of D_4 receptor on AT_1 receptor expression in WKY RPT cells was blocked by a calcium channel blocker, nicardipine (10^{-6}M) or calcium free medium, indicating that calcium channel was involved as a signaling molecule in the D_4 receptor-mediated signaling pathway. Angiotensin II (10^{-11}M) increased Na^+-K^+ ATPase activity in WKY cells. Pretreatment with PD168077 (10^{-6}M/24hrs) decreased the stimulatory effects of angiotensin II on Na^+-K^+ ATPase activity in WKY cells. However, in SHR cells, the inhibitory effect of D_4 receptor on angiotensin II-mediated effect on Na^+-K^+ ATPase activity